Certificate of Analysis

Mate & Plate™ Library – Mouse Embryonic Stem Cell (Normalized)

Catalog No.	Lot Number
630484	1904885A

Description

This yeast two-hybrid library was constructed from mRNA isolated from mouse embryonic stem cells (E14TG2A cell line) and transformed into yeast strain Y187. The cDNA was normalized prior to library construction to reduce the copy number of abundant cDNAs derived from highly represented mRNAs, thereby increasing the representation of low copy number transcripts. The normalization process combines a Duplex-Specific Nuclease (DSN) treatment and SMART™ technology, reduces the number of clones that must be screened in your yeast two-hybrid assay, and facilitates the identification and characterization of novel protein-protein interactions.

The library was transformed into yeast strain Y187 and can be readily mated to a *MATa* GAL4 reporter strain, such as AH109 or Y2HGold, for screening.

Package Contents

- 5 x 1.0 ml Mate & Plate Library - Mouse Embryonic Stem Cell (Normalized)
- 1 x 1.0 ml Mate & Plate Library - Control (pGADT7-T in Y187)

Storage Conditions

- Store all components at –70°C
- Do not refreeze

Shelf Life

- 1 year from date of receipt under proper storage conditions.

mRNA Source

- Poly A+ RNAs isolated from mouse embryonic stem cells (E14TG2A cell line).

Cloning Vector

- pGADT7-RecAB

Cloning Site

- Sfi I A/Sfi I B

Priming Method

- Sfi I $(dT)_{30}$ primed

Yeast Genotype

- (Y187): MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, gal4Δ, gal80 Δ, met–, URA3 :: $GAL1_{UAS}$-$GAL1_{TATA}$-LacZ, MEL1

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Shipping Conditions

- Dry ice (–70°C)

Product Documents

Documents for our products are available for download at **takarabio.com/manuals**
The following documents apply to this product:

- Matchmaker™ Gold Yeast Two-Hybrid User Manual (PT4084-1)
- pGADT7-RecAB Vector Information (PT3718-5)

Quality Control Data

1. Quality Control Data

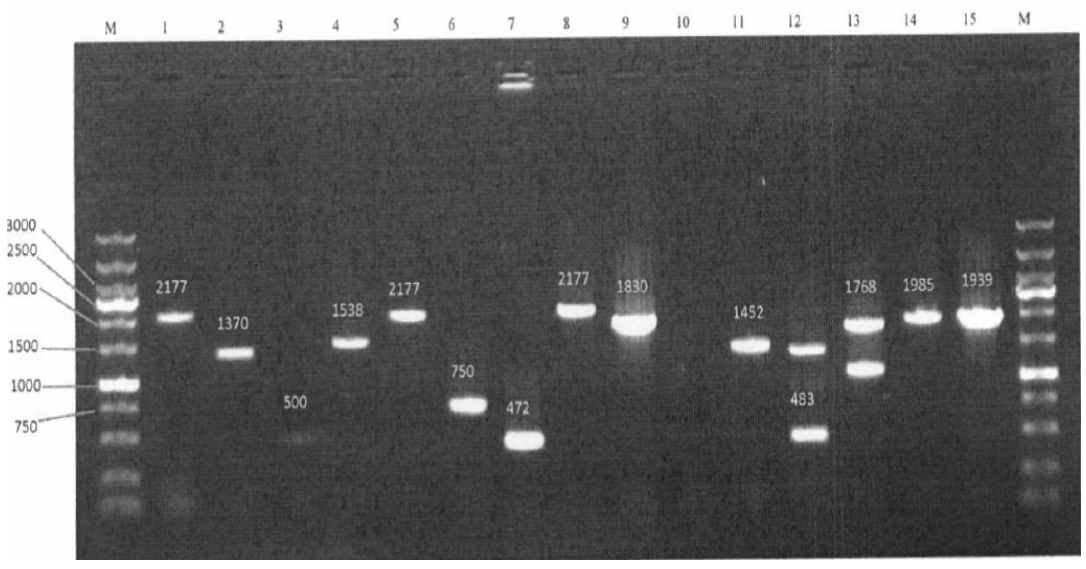
Test	Result
Titer (yeast colonies)	>5 x 10^7 cfu/ml
Number of independent clones	1.5 x 10^6
Average cDNA size	1.5 kb
cDNA size range*	0.4-2.1 kb

*the cDNA was size-selected by excision from an agarose gel prior to cloning

2. Quality Control Data for the Pretransformed Library in Yeast
   Library Insert Size Screening
   15 yeast colonies were randomly picked and screened by PCR using the Matchmaker AD LD-Insert Screening Amplimer Set (Cat. No. 630433).

   14 of 15 colonies contained inserts as determined by PCR.

Lane 1. 2.18
2. 1.37
3. 0.50
4. 1.54
5. 2.18
6. 0.75
7. 0.47
8. 2.18
9. 1.83
10. -
11. 1.45
12. 0.48
13. 1.77
14. 1.99
15. 1.94



3. cDNA Normalization

cDNA generated using SMART technology was normalized using Duplex-Specific Nuclease (DSN) normalization (Shagin et al., 2002; Zhulidov et al., 2004). Prior to cloning, the efficiency of normalization was assessed by virtual Northern blot analysis (Franz et al., 1999) comparing the abundance of β-actin and GAPDH in normalized and non-normalized mouse embryonic stem cell cDNA (Figure 1).

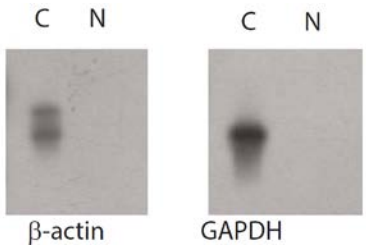


Figure 1. DSN-Normalization reduces the amount of highly abundant transcripts. Normalized (Lanes N) and non-normalized (Lanes C) mouse embryonic stem cell cDNA samples (PCR products) were electrophoresed on a 1.5% agarose gel and transferred to Hybond-N membrane. PCR-amplified probes of β-actin and GAPDH were labeled with ^{32}P-dATP and hybridized to the membrane. GenBank Accession numbers: β-actin, NM_001101 and GAPDH, NM_02406.

4. Embryonic Stem Cell Confirmation

The mRNA from mouse ES cells expresses 4 pluripotency-related genes, Nanog, Oct-4, Klf-4, and Sox-2 (Figure 2).

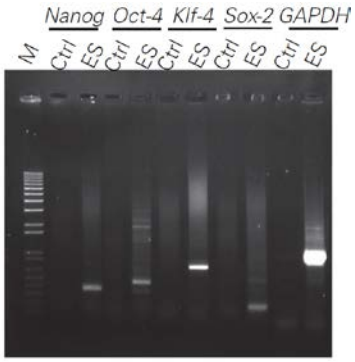


Figure 2. ES cells express pluripotency-related markers. mRNA from embryonic stem cells (ES) (E14TG2A cell line) were analyzed for gene expression markers associated with pluripotent (stem) cells. M, molecular weight marker (1 kb Plus DNA ladder.)

References

Franz, O., Bruchhaus, I., & Roeder, T. (1999). Verification of differential gene transcription using virtual northern blotting. *Nucleic Acids Research*, *27*(11), 3e – 3. https://doi.org/10.1093/nar/27.11.e3

Shagin, D. A., Rebrikov, D. V, Kozhemyako, V. B., Altshuler, I. M., Shcheglov, A. S., Zhulidov, P. A., Lukyanov, S. (2002). A Novel Method for SNP Detection Using a New Duplex-Specific Nuclease From Crab Hepatopancreas. *Genome Research*, *12*(12), 1935–1942. https://doi.org/10.1101/gr.547002

Zhulidov, P. A., Bogdanova, E. A., Shcheglov, A. S., Vagner, L. L., Khaspekov, G. L., Kozhemyako, V. B., Shagin, D. A. (2004). Simple cDNA normalization using kamchatka crab duplex-specific nuclease. *Nucleic Acids Research*, *32*(3), 37e – 37. https://doi.org/10.1093/nar/gnh031

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

Notice to Purchaser

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NOTICE TO PURCHASER:

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

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